

# Novel Fastidious, Partially Acid-Fast, Anaerobic Gram-Positive Bacillus Associated with Abscess Formation and Recovered from Multiple Medical Centers

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**We report a novel anaerobe causing abscess in four patients at three hospitals. In the clinical specimen, bacilli were branching, Gram positive, and acid fast. The organism grew slowly and was not identified by 16S rRNA sequencing. Our findings support the description of a new genus and species of the suborder *Corynebacterineae*.**

## CASE REPORTS

**Case 1.** A 65-year-old male with metastatic prostate cancer diagnosed in Cleveland, OH, in March 2009 presented 1 June 2011 with an enlarging mass adjacent to a surgical incision at T3-8 from previous spinal stabilization at the time of diagnosis. Medications included prednisone (5 mg daily), docetaxel, zoledronic acid, and leuprolide. There was no significant travel history, exposure to animals, or sick contacts. He denied trauma to the upper back and had a sedentary life. A physical exam revealed a weight of 130 kg, blood pressure of 106/78, pulse of 160/min (atrial fibrillation), temperature of 38.6°C, and respiratory rate of 22/min. He was unaware of his fever. The mass was minimally warm to the touch, mildly erythematous, fluctuant, nondraining, and non-painful. The white blood cell (WBC) count was 12,370/ $\mu$ l with 77% neutrophils.

The magnetic resonance imaging (MRI) and computed tomography (CT) scan were consistent with thoracic fluid collection and abscess. Surgical incision and drainage produced a large amount of purulent, nonodorous fluid. Pedicle screws placed previously were solidly in position, and there was no evidence of bony destruction. Partial hardware removal and debridement of necrotic tissue were performed. A swab of the subfascial tissue, fluid, and hardware were submitted for culture. A beaded Gram-positive, branching bacillus and inflammatory cells were present on Gram stains of all material submitted. Modified Kinyoun stain was positive. (Fig. 1a and b).

Vancomycin started prior to surgery was switched to ampicillin-sulbactam but changed to meropenem due to suspicion of *Nocardia*. Blood cultures produced no growth. The white blood cell count normalized, and he was afebrile by postoperative day 2. His surgical site healed well, and he was discharged postoperative day 7 on meropenem. After 4 weeks, home antibiotic therapy was switched to sulfamethoxazole-trimethoprim (SXT) at 800 to 160 mg with two tablets 3 times per day (TID). Docetaxel chemotherapy was resumed.

Anaerobic cultures of the fluid and hardware grew after 13 days of incubation. Cultures performed from swabs of tissue did not grow, nor was growth obtained from any specimen submitted for

culture for mycobacteria, fungi, and aerobic bacteria. By partial 16S rRNA sequencing, the organism was <95% identical to *Dietzia*, *Tsukamurella*, and *Corynebacterium* spp. The 16S rRNA sequence (1,445 bp) was later submitted to GenBank. With these results, therapy was changed to amoxicillin-clavulanate (1,000/62.5 mg twice daily).

Five weeks postoperatively, a scant amount of clear yellow nonodorous fluid was noted draining from a pinhole-sized opening of a 3-cm fluctuant, nonerythematous collection at the surgical site. He denied any pain. Fluid was not submitted for culture at this time, and scant fluid continued to drain intermittently. At 6 and 10 months postsurgery, 50 to 100 ml of serous fluid was drained from a palpable seroma. Cultures collected 3 and 6 months postsurgery were negative for bacteria, despite prolonged incubation under anaerobic conditions. He remained on amoxicillin-clavulanate until he expired from complications of metastatic prostate cancer in October 2012.

**Case 2.** A 44-year-old obese female presented in Winnipeg, Manitoba, in July 2012 with tender induration of the left breast. Past medical history included a dermoid cyst of the ovary, steatosis of the liver, a recent diagnosis of diabetes mellitus, and a left breast abscess in 2003 that was surgically drained and managed with a short course of antibiotic therapy. However, culture results were not available for that episode. On 13 August 2012, an incision and drainage were performed, at which point, the surgeon noted a large amount of pus in a loculated abscess. Loculations were removed, and the abscess cavity was curetted out. Specimens were submitted for histopathology and culture. Histopathology showed inflammatory tissue and fibrosis compatible with abscess. No special stains were performed. Direct Gram stain revealed a

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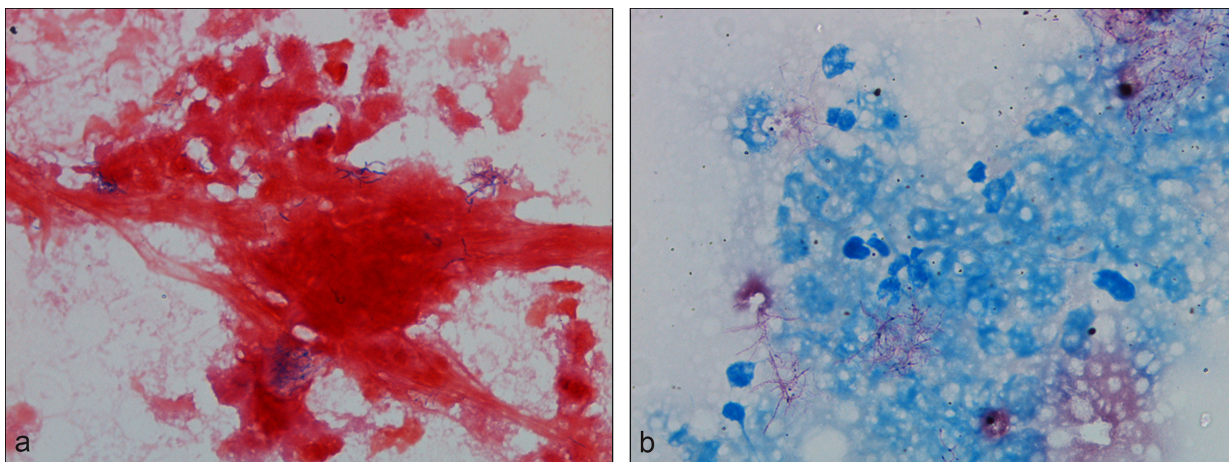


FIG 1 Clinical specimen from spinal abscess (case 1; CCF-01). (a) Gram stain,  $\times 500$  magnification; (b) modified Kinyoun stain,  $\times 1,000$  magnification.

large amount of pus and a large amount of branching Gram-positive rods, which were acid fast by the Kinyoun method. The specimen was planted for mycobacterial, routine aerobic and anaerobic, and fungal culture. On the fifth day of incubation, a heavy amount of fine growth was noted on anaerobic cultures. All other cultures were negative. Amplification and sequencing of an 800-bp fragment of the 16S rRNA gene using primers 8FPL (5'-A GTTTGATCCTGGCTCAG-3') and 806R (5'-GGACTACCAGG GTATCTAAT-3') was performed. A 773-bp segment was compared against the GenBank (NCBI) and Ribosomal Database Project (RDP; Michigan State University) databases, which demonstrated that the segment was 100% identical to the recently submitted sequence of bacterium CCF-01. This strain had been frozen at  $-70^{\circ}\text{C}$  in skim milk. However, following the sequencing results, it was not further characterized as it could not be recovered from the frozen stock culture. The patient was treated with vancomycin for 2 days, briefly switched to cloxacillin, and placed on amoxicillin-clavulanate on 16 August 2012. There was good initial clinical response, and she remained on amoxicillin-clavulanate at 500/125 mg TID for a period of 6 months. At last follow-up, the abscess was completely resolved with no evidence of recurrence.

**Case 3.** A 23-year-old female presented in Winnipeg, Manitoba, in July 2012 with a 3-month history of a progressively enlarging erythematous left breast lump. She had poorly controlled type II diabetes, recurrent diabetic foot ulcers, and recurrent furunculosis, including a remote superficial left breast abscess that required incision and drainage and grew *Staphylococcus aureus*. The current lesion developed while the patient was receiving ciprofloxacin and metronidazole for a diabetic foot infection with chronic osteomyelitis. At incision and drainage, purulent material was recovered from a superficial abscess cavity. She was continued on ciprofloxacin and metronidazole and completed therapy on 10 February 2013. She also received a 2-week course of SXT for an episode of methicillin-resistant *Staphylococcus aureus* (MRSA) furunculosis on her back in October 2013. Erythema and induration slowly improved after drainage, and there was no sign of active infection at follow-up in October.

Gram stain of the aspirate from the breast abscess revealed heavy pus and weakly Gram-positive, refractile, branching rods. A Kinyoun acid-fast stain demonstrated a large amount of acid-fast

bacilli. Anaerobic incubation for 4 days yielded a moderate amount of growth. There was no growth from aerobic, fungal, and mycobacterial cultures. Sequencing of the 16S rRNA gene revealed 100% sequence identity to bacterium CCF-01 in GenBank. The isolate was submitted to the National Microbiology Laboratory (NML), Public Health Agency of Canada, in Winnipeg, Manitoba, Canada, for further characterization.

Cases 2 and 3 were identified in the same laboratory. Investigation into possible relationships between cases 2 and 3 was conducted. They did not reside in close proximity and received medical care at different sites in Winnipeg, Manitoba, Canada. Both cases had previous breast abscesses, but medical procedures were performed at different locations.

**Case 4.** An 81-year-old male presented to a gastroenterologist in New York in November 2012 with elevated liver enzymes and intermittent fevers for several weeks. Past medical history included polymyalgia rheumatica, currently treated with prednisone at 5 mg orally (p.o.) daily for over 1 year, a 4-vessel coronary artery bypass graft in 1998, an automatic implantable cardiac defibrillator (AICD) in 2010, and aortic stenosis with aortic valve replacement in 2011. A CT scan of his abdomen and pelvis revealed a hypodense mass (4.8 by 3.8 by 3.5 cm) in the left lobe of the liver with poor enhancement, suspicious for malignancy. A 5 December 2012 liver biopsy specimen demonstrated necrosis and inflammation with plasma cells and neutrophils. No malignancy was seen. Culture was not obtained at the time.

Laboratory tests revealed a WBC count of  $10,600/\mu\text{l}$  with 9% bands and 80% neutrophils. The erythrocyte sedimentation rate (ESR) (100 mm/h) and C-reactive protein (CRP) level (7.4 mg/dl) were elevated. Other significant laboratory values included elevated globulins (4.3 g/dl), aspartate aminotransferase (AST) (57 IU/liter), alanine aminotransferase (ALT) (71 IU/liter), alkaline phosphatase (ALK) (99 IU/liter), and lactate dehydrogenase (214 IU/liter) and decreased hemoglobin (9.6 g/dl) and albumin (3.0 g/dl). A Quantiferon Gold assay was indeterminate, and an amplified *Mycobacterium tuberculosis* direct test on the aspirate was negative.

He continued to have intermittent fevers of  $38.9^{\circ}\text{C}$ . On 27 December 2012, a repeat aspirate of the liver produced purulent material. Direct Gram stain showed numerous white blood cells and few filamentous, branching Gram-positive rods. The rods

stained acid-fast positive by the modified Kinyoun method. Histopathology showed abundant acute inflammation, consistent with abscess. Aspirate material was sent to the University of Washington, Seattle, for 16S rRNA gene sequencing, with 100% sequence identity to the sequence of bacterium CCF-01 deposited in GenBank. Nontuberculous mycobacterial DNA was not detected by PCR with 16S rRNA, *hsp65*, and *rpoB* primer sets.

Given the Gram stain result and clinical status, he was empirically treated with levofloxacin, metronidazole, vancomycin, clindamycin, and SXT. He was discharged home on clindamycin and SXT with suspicion of *Actinomyces* or *Nocardia* infection. The aerobic cultures were negative at 21 days. The anaerobic cultures, held for 5 days, were also negative. The fungal and mycobacterial cultures remained negative.

Intermittent fevers continued. A repeat abdominal CT on 10 January 2013 revealed multiple irregular 4- to 5-cm fluid collections within and anterior to the left lobe of the liver that were consistent with abscesses. Two drains were placed in the largest abscesses on 16 January. A repeat aspirate produced 50 ml of cloudy light brown fluid, and cytology revealed acute inflammation and debris. Gram stain of the second aspirate showed numerous white blood cells but no organisms. Bacterial, fungal, and mycobacterial cultures of the material were negative. Routine blood cultures were negative. The patient was discharged home on oral SXT (two double-strength tablets, 2 times daily) with drains in place. By 23 January, the patient was afebrile and feeling better. A repeat abdominal CT showed that the two abscesses were almost resolved, and the other undrained liver abscesses were slightly smaller. The patient remained afebrile but with continued mild right upper quadrant discomfort. Repeat CT on 7 March showed a 1.5- by 1.4-cm recurrent perihepatic collection above the left lobe of the liver, while the intrahepatic abscesses resolved on SXT. Repeat CT on 2 April showed persistent perihepatic collection, which was drained, releasing very thick, foul-smelling fluid. SXT was stopped on 2 April 2013. Laboratory values remained largely unchanged, with an elevated ALT of approximately 100 IU/liter, and an elevated AST of 75 IU/liter, ALK of 140 IU/liter, CRP of 12 mg/dl, and ESR of 130 mm/h. His peripheral WBC count was normal.

**Microbiology.** Obtaining growth from both primary culture and subculture was somewhat challenging. The first isolate (case 1) grew on CDC anaerobic blood agar (CDCAB) (BBL, Becton Dickinson [BD], Sparks, MD) at 35°C using a gas pack (GasPak EZ#260678; Becton Dickinson) in an anaerobe jar after 13 days. Isolates from cases 2 and 3 grew on brucella agar with vitamin K (BBL) after 5 and 4 days, respectively, using a gas pack (Oxoid AnAerogen, Basingstoke, United Kingdom) in an anaerobe jar. There was no growth in fastidious anaerobic broth (FAB; LabM, Lancashire, United Kingdom). Colonies were pinpoint, white, and waxy. In each case, pure growth was obtained. The organism from case 4 never grew *in vitro*.

Although bacilli from clinical material were positive with the Kinyoun stain, on subculture, they were only partially acid fast by the modified Kinyoun staining procedure. The morphology of the organisms from colonies was small, nonbranching rods. On subculture, CCF-01 grew well on CDCAB incubated anaerobically at 35°C, but it also grew anaerobically on Trypticase soy agar II (TSA II) with 5% sheep blood (BBL). CCF-01 grew on subculture under microaerophilic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>) at 35°C on CDCAB, but growth was very poor and could not be sustained

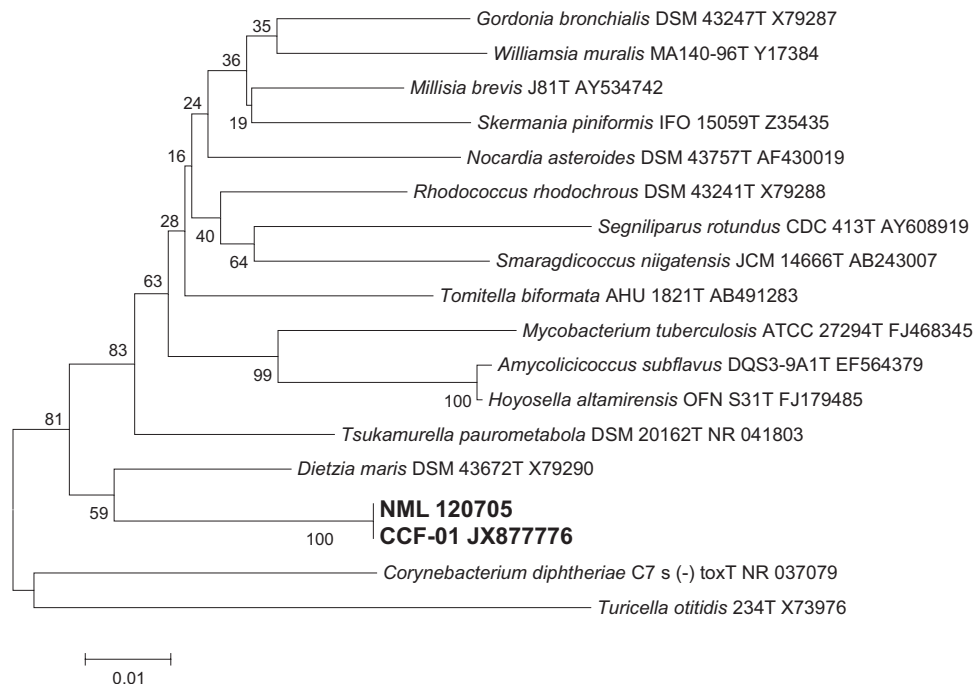
in 5% CO<sub>2</sub>. There was no growth on buffered charcoal yeast extract agar (BCYE), chocolate agar, chocolate agar with olive oil, potato dextrose agar, and Middlebrook 7H11 agar incubated anaerobically at 35°C. There was no growth on any medium in the room air environment. The need for prolonged incubation limited laboratory characterization, and susceptibility testing could not be performed.

The organism from case 1 was referred to the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, for additional testing. Growth studies, modified acid-fast staining, and 16S rRNA sequencing (1,445 bp) were confirmed at the CDC. They found sustained growth to be best with subculture to CDCAB and incubation at 35°C using a gas pack in an anaerobe jar. The GasPak system generates an environment with <1% oxygen and ≥13% CO<sub>2</sub>. Attempts to grow the organism in an anaerobic chamber at the CDC were unsuccessful. Repeated attempts to grow the organism in liquid media have been unsuccessful. The following liquid media were utilized: Trypticase soy broth, heart infusion broth, *Haemophilus* test medium, and brucella broth. The organism could be maintained in enriched thioglycolate broth (BBL), but no increase in cell mass was detected.

Laboratory assessment of the isolate from case 3 was performed at the NML (identifier no. NML 120705). The organism grew on brucella blood agar (BBA) (BBL) incubated anaerobically at 35°C, but it did not grow under other conditions. There was no growth on chocolate agar, BCYE with and without cysteine, MK7H10, or Columbia blood agar under anaerobic conditions. The bacterium appeared to grow better when a *Staphylococcus aureus* streak was added to BBA. The organism did not grow in PY (peptone-yeast extract) broth alone or augmented with dextrose, serum, Tween 80, bile, or formate-fumarate. No growth or negative results were obtained for a panel of more than 30 PY sugars and substrates used for identification of anaerobes (1). No growth was obtained with an API 32A panel (bioMérieux). Cellular fatty acid (CFA) analysis was performed in duplicate, as described previously, except that growth from 5 BBA plates was harvested after 5 days of growth anaerobically at 35°C, combined into 1 broth containing PY with dextrose plus Tween, incubated for 48 h, and then extracted (1). For each attempt, the organic phase required further concentration to bolster counts. The CFAs detected based on two runs were C<sub>10:0</sub>, C<sub>14:0</sub>, C<sub>16:1ω7</sub>, C<sub>16:0</sub>, C<sub>17:0</sub>, C<sub>18:2ω6,9</sub>, C<sub>18:1ω9</sub>, and C<sub>18:0</sub> after manipulation of raw data to accommodate for low counts; C<sub>10me17:0</sub>, and particularly tuberculostearic acid (TBSA; C<sub>10me18:0</sub>), a fatty acid often found in small to large quantities among genera of the suborder *Corynebacterineae*, were not detected. 16S rRNA gene sequencing was performed as described previously, and a 1,454 bp product was deposited in GenBank (1). An alignment of 16S rRNA sequences of CCF-01 and NML 120705 compared to members of the suborder *Corynebacterineae* was generated using ClustalW and the neighbor-joining algorithm from MEGA5 (Fig. 2).

In this report, we have described four cases of monomicrobial abscess in patients from Cleveland, OH, Winnipeg, Manitoba, Canada, and New York City. This novel agent grew anaerobically, and abscesses were found near the spine with associated hardware, in breast tissue, and in the liver. Abscess formation was the common feature but not the anatomic site or underlying illness. Ab-





**FIG 2** 16S rRNA alignment of the suborder *Corynebacterineae*, illustrating the novel status of NML 120705 and CCF-01 (boldface). Alignment was done using ClustalW, and relationship was inferred using the neighbor-joining algorithm from MEGA5.1. The scale represents 0.01 substitution per nucleotide position. The type species of each genus in the suborder is shown, with *Dietzia* as the closest genus.

cesses with anaerobes are frequently polymicrobial and are often mixed with aerobic species. Oddly, although anaerobic, the etiologic agent in our cases is more closely related to the aerobic actinomycetes than to other anaerobes. While mycobacteria and *Nocardia* spp. may be associated with abscess formation, abscess due to other aerobic actinomycetes is more unusual as most of these species are relatively infrequent human pathogens. However, reports include at least brain, soft tissue, liver, and lung abscesses with *Rhodococcus* spp. (2–5) and breast and other tissue abscesses with several *Corynebacterium* species (6). Infections with *Dietzia* spp. are rarer but include bacteremia and infections of a prosthetic joint and a pacemaker pocket (7–9).

Based on identical 16S rRNA sequence (100%), CCF-01, NML 120705, and the organisms from the other two cases are likely the same genus and species. However, they appear to be a novel genus, as the closest related genus, *Dietzia*, had <95% similarity. The alignment of nearest neighbors demonstrates the limited relatedness to members of the suborder *Corynebacterineae*, and the cellular fatty acids detected are suggestive of various Gram-positive bacteria and fit members of the genus *Dietzia*, with the exception of the absence of  $C_{10me17:0}$  and  $C_{10me18:0}$  (10).

The growth and staining characteristics are features of this species that are completely novel. We know of no description in the medical literature of an anaerobe that is closely related to the aerobic actinomycetes or one that stains with the modified Kinyoun stain. Primary growth was always obtained under anaerobic conditions. However, some aerotolerance was noted, as CCF-01 grew in microaerophilic conditions upon subculture. This species grew on brucella blood agar and CDC anaerobic blood agar but not on Middlebrook 7H11 agar, a medium that supports aerobic actinomycetes. Growth was also not obtained on either chocolate agar or

BCYE, which allow the growth of most fastidious organisms. We speculate that this species has a requirement for hemin and vitamin K, as these components are enriched in the media on which CCF-01 was cultivated. Although growth could be obtained on TSA II, investigators at the CDC have observed that growth is supported inconsistently on subculture.

Some results remain puzzling. Using media available in clinical laboratories, about 2 weeks was needed to obtain growth from primary cultures of case 1. The more rapid growth for cases 2 and 3 may be due to the presence of a very large organism burden, as longer incubation was needed on subculture. Alternatively, the presence of a critical growth factor (as yet unidentified) in the clinical material itself could have enhanced the growth of the organism. Interestingly, growth did not occur in the anaerobic broths incubated with the clinical specimens from cases 2 and 3. This may support the hypothesis that something in the clinical material, such as a fatty acid, facilitated more rapid growth when the specimen was placed on solid medium. We noted that the organism was isolated from areas of fatty breast tissue in two of our patients, from fatty liver, and from soft tissue in an obese patient. However, there was also no growth in Middlebrook 7H9-based broths used for routine mycobacterial cultures (BD MGIT [cases 1 and 4] and bioMérieux BacT/Alert MP [cases 2 and 3]). Neither the oleic acid added as part of the oleic acid-albumin-dextrose-catalase (OADC) 7H9 broth supplement nor olive oil added to chocolate agar was sufficient to promote growth. The presence of a required growth factor in the specimen or resistance to decolorization due to a purulent sample could also explain acid-fast properties observed on direct stain but not on subculture. Branching was also seen on primary stains, but not on sub-

culture, further reflecting differences in growth *in vivo* versus on available laboratory media.

Due to growth limitations, specifically in liquid media, no susceptibility testing has been performed to date, and the best therapy is still unknown. However, studies with different media are ongoing. Based on the original Gram stains, two patients (spinal and liver abscess) were initially treated with therapy for *Actinomyces* and *Nocardia*. Patients with spinal abscess and breast abscess (case 2) improved on amoxicillin-clavulanate, which has activity for most anaerobes. Although fluid continued to drain from the wound of case 1, the wound did not cause significant morbidity within the context of his prostate cancer. The hardware remaining in his spine could have contributed to biofilm production that was never completely eliminated. The patient from case 4 showed improvement on SXT, an antibiotic combination not usually recommended for antianaerobic therapy. One patient with breast abscess improved while undergoing therapy with ciprofloxacin and metronidazole for a diabetic foot infection and also received a short course of SXT. As with most abscesses, incision and drainage likely made a significant contribution to recovery in each patient.

An environmental niche for this microorganism is unknown. It is possible that this organism is an unrecognized species within the human microbiota, but this seems less likely than acquisition from the environment. Two of the patients were on steroids and were mildly immunosuppressed, a third patient had poorly controlled diabetes, and the fourth had recently diagnosed diabetes. These underlying conditions may have contributed to acquisition of the organism and promoted pathogenicity in these hosts. We speculate that it is a relatively low-virulence species, as all of the patients improved with drainage and/or therapy, and none had more invasive disease.

To the best of our knowledge, this organism has not been previously described, but it has now been detected in three geographically separate locations. Complete chemotaxonomic characterization and genome sequencing are in progress at the Leibniz-Institut DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany) and CDC, respectively. These additional data may help us understand the growth requirements of this species and relationships to others. Based on the data presented herein, we believe this to be a novel genus and species that may be in the suborder *Corynebacterineae*. However, although this species has been isolated from multiple patients with clinically significant infection, we have foregone proposing a genus and species name until chemotaxonomic characterization is

complete and suggest this microbe be designated CCF-01 in the interim. We believe that insufficient data are currently available to determine the correct taxonomic family.

**Nucleotide sequence accession numbers.** The 16S rRNA sequence (1,445 bp) from case 1 has been submitted to GenBank as bacterium CCF-01 (accession no. [JX877776](#)). The 16S rRNA sequence (1,454-bp product) from case 3 (NML 120705) has been deposited in GenBank under accession no. [KC669624](#).

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